Effect of Indole-3-Acetic Acid on Tensile Creep of Japanese Black Pine Hypocotyl

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Kazuo SUMIYA* and Tadashi YAMADA*

Abstract——Tensile creep experiments were employed to acertain the effect of indole-3-acetic acid (IAA) on the extensibility of the cell walls of woody plants. Hypocotyls of Japanese black pine in their rapid growth stage were used. It was determined that 10 ppm IAA changed the tensile creep process to the one which was difficult to elongate at later stage. It was also suggested that 10 ppm IAA increased the retarded elasticity and inhibited the flow which occurs under a small load without IAA. An other result was that the elongation of the living segment was sensitive to the load previously added to it.

Introduction

In the preceding paper[1] we reported that 10 ppm indole-3-acetic acid (IAA), which was applied to rapidly growing seedlings of Japanese black pine, changed hypocotyl's relaxation process to one which was difficult to relax at the last stage. We also suggested that this process, which mainly contributed to YOUNG's modulus of the hypocotyl segment, seems to be concerned with the maturation of the cell wall. Effort has been made to clarify the effect of IAA to the extensibility of the woody cell wall. Because the extension of the cell wall during growth occurs under a given osmotic pressure, creep experiment is useful for examining the elongation process of the cell wall. In the present paper, accordingly, we describe the creep behavior of the segment under tension.

Materials and Methods

Wood Materials: Japanese black pine seedlings (*Pinus thembergii* PARL.) were grown on vermiculite for 2 days after germination under 20000 lux artificial light radiation of 14 hr/day in a growth cabinet conditioned at 28°C and 85% relative humidity. These seedlings were in a rapidly growing stage.

Subsequently, the hypocotyl of each seedling were soaked in water or 10 mg/liter IAA for 2 days under the same condition as mentioned above before a segment, 10 mm long, was excised from the upper portion of the hypocotyl. Three of these excised segments were immediately subjected to tensile creep test followed by creep recovery test in 0.25 mol mannitol solution to examine the behavior of the living segment preventing the cell wall extension by the osmotic pressure. The other three

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segments were killed in hot methanol and then subjected to the same test in water to examine the behavior of the cell wall itself.

Creep test: A tensile creep tester with a recorder was used at 20°C. In order to minimize the obstructive impact against the sample, a constant tensile load was added on the sample by a given amount of silicon oil which was dropped from a pipet, and was removed by an aspirator.

The constant tensile stress used in these experiments were 1.7 and 0.30 kg/cm^2 for the living and the killed segment respectively. These stresses strained the sample to elongate about 2 %, which was of the same order of strain as in the preceding stress relaxation experiments and was within the elastic range of stress-strain curve.

The creep and creep recovery curves for 200 min. each were automatically recorded through a linear transducer to give the elongation- and contraction-time curves.

If a constant stress σ_0 is put on the sample at time $t=0$, the strain, ε, as a function of time will be

$$\varepsilon(t)=\sigma_0 J(t) \qquad (1),$$

where $J(t)$ is the creep compliance. And if this constant stress is removed at $t=t_1$, the total strain, $\varepsilon_r(t)$, thereafter will be

$$\varepsilon_r(t)=\sigma_0 J(t)-\sigma_0 J(t-t_1) \qquad (2),$$

according to the BOLTZMANN superposition principle.

On the other hand, the creep compliance, $J(t)$, can be described as follow:

$$J(t)=J(0)+(J_e-J_0)\Psi(t)+\phi(t) \qquad (3),$$

where J_e and J_0 are the equilibrium and limiting compliance, $\Psi(t)$ is the retarded-elasticity function increasing monotonically from 0 to unity, and $\phi(t)$ is the function concerned with flow.

Then, the creep strain at $t=t_1$ can be described as

$$\varepsilon(t_1)=\sigma_0[J(0)+(J_e-J_0)\Psi(t_1)+\phi(t_1)] \qquad (4),$$

and the strain after removal of load can be also described as

$$\varepsilon(t)=\sigma_0[(J_e-J_0)\{\Psi(t)-\Psi(t-t_1)\}+\{\phi(t)-\phi(t-t_1)\}] \qquad (5).$$

The difference between eqs. (4) and (5), that may be called the recovery strain, $\varepsilon_{rec}(t)$, will become

$$\varepsilon_{rec}(t)=\sigma_0[J(0)+(J_e-J_0)\Psi(t-t_1)+\phi(t-t_1)]+\sigma_0[\phi(t_1)-\phi(t)] \qquad (6),$$

if the times, t_1 and t, are long enough for the retarded-elastic strains to reach their equilibrium values. The first term of the right side of this equation is equivalent to the creep strain started at time $t-t_1$. So, the shape of the function concerned with flow, $\phi(t)$, can be estimated experimentally from the difference between the creep compliance and the mirror image of this recovery compliance displaced vertically by $J(0)$ and horizontally by t_1 and reflected in the time axis, whether the flow is Newtonian or not.

Results and Discussion

Creep compliance data, subtracted the instantaneous creep compliance, which were obtained from the creep and the mirror image of creep recovery of the living segments are shown in Fig. 1. The averages of the instantaneous tensile strain were 1.81, 1.56, 2.54 and 2.14% for the living segments grown with or without IAA and the segments killed by hot methanol after the treatment with or without IAA, respectively. Both these values of the instantaneous strain and the creep compliance curves obtained from the creep test in Fig. 1 indicated that the application of 10 ppm IAA solution increased the creep compliance at early stage, and decreased it at later stage, comparing with the case without IAA. The analogous action of IAA on pine seedlings was already found in the preceding stress relaxation experiments[1].

A very queer phenomenon could be seen in the curve obtained from creep recovery test as shown in Fig. 1. The creep compliance began to decrease after the elapsed time of about 50 min. This means that the living segments, which has been supplied a given load for a long time and has elongated to some extent, can begin to elongate again without any osmotic pressure at a time after removal of load. This fact may suggest that the elongation of the living segment is sensible to the load previously added to it. This fact essentially brought a difficulty into the

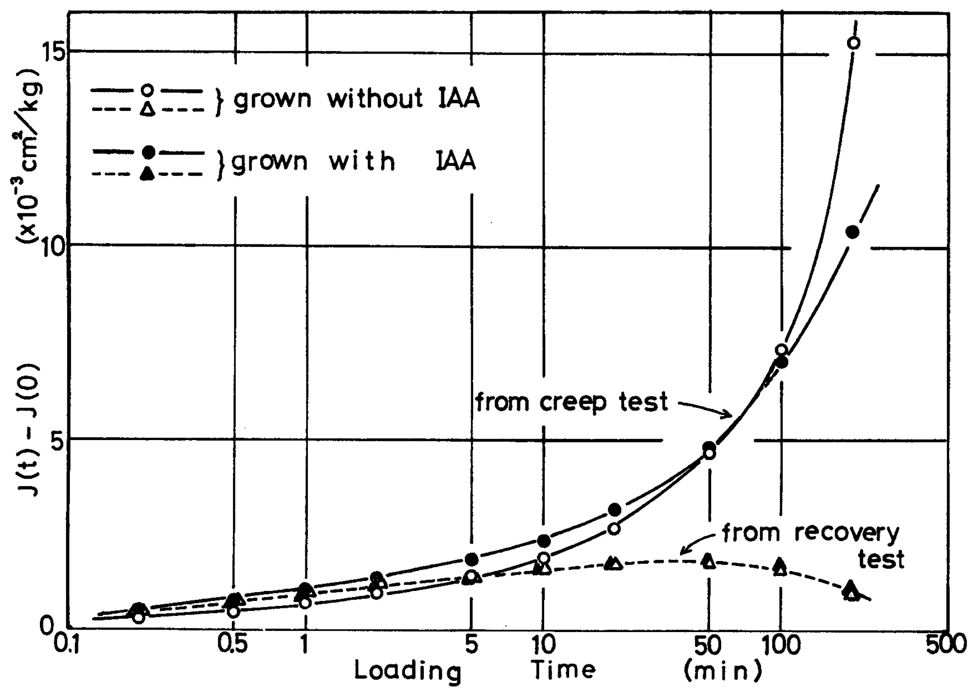

Fig. 1. Creep compliance curves at 20°C for the living segments.

estimation of the shape of the function concerned with flow caused by the external force on the living segment. This difficulty is attributable to the inapplicability of the BOLTZMANN superposition principle, namely eq. (2), and then eq. (6).

Creep compliance data, subtracted the instantaneous creep compliance, which were obtained from the creep and the mirror image of creep recovery of the killed

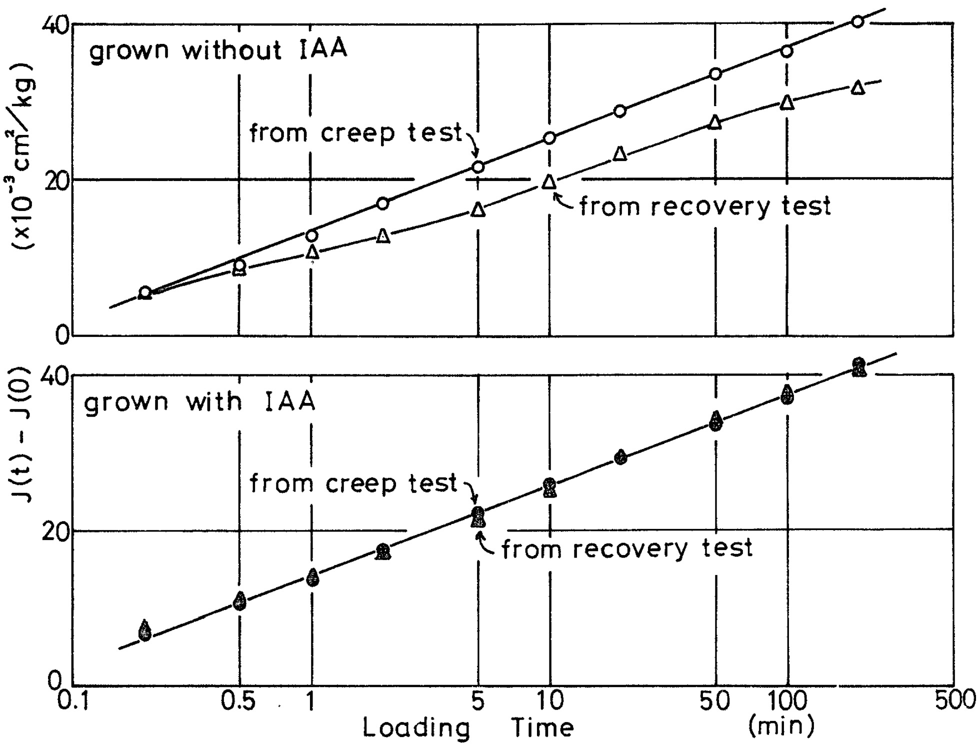

Fig. 2. Creep compliance curves at 20°C for the segments killed by hot methanol.

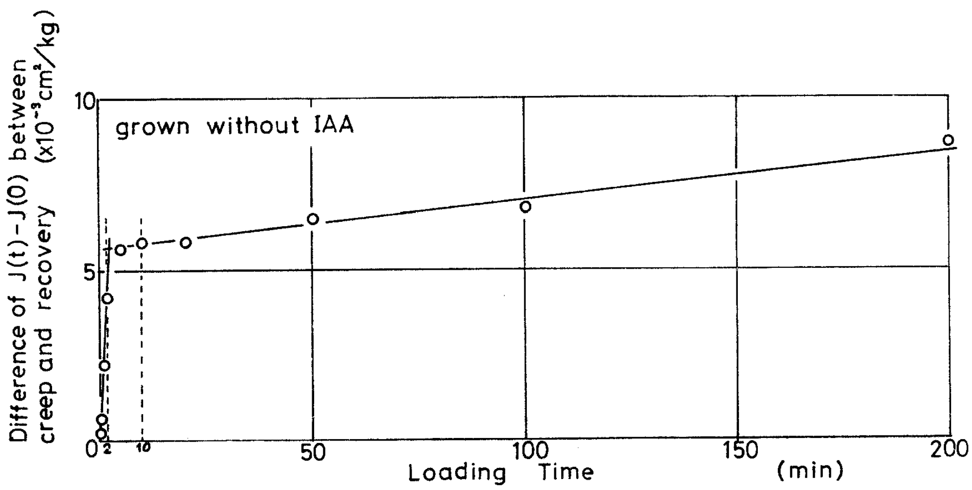

Fig. 3. The time-dependency of the difference between the compliances obtained from creep test and from creep recovery test in Fig. 2. This difference is concerned with flow occurring in the segments. See eq. (6) and text.

segment are shown in Fig. 2. The value of instantaneous creep compliance was much increased by methanol treatment, corresponding to the lowering of YOUNG's modulus by this treatment[1]. Also the rapid rise of creep compliance, which was found near the lapsed time of about 50 min. in the case of living segment, went out of the time range of observation, in analogous to the delay of rapid fall of stress relaxation found in the preceding experiments[1].

It was the effective case of the BOLTZMANN superposition principle, and the shape of flow would be able to be estimated experimentally, as described in the previous section. In the cell wall of the segments grown without IAA, the flow was non-Newtonian, but might be of the type that the coefficient of viscosity in Newtonian flow would change to the larger one with the lapse of time of from 2 to 10 min., as shown in Fig. 3. In the cell wall of the segment grown with IAA, on the other hand, creep compliances obtained from creep and creep recovery test coincided with each other, as shown in Fig. 2. This fact indicates that there is no flow in the cell wall of these segments, according to eq. (6). It seems to be certain that one of the actions of 10 ppm IAA solution on the cell wall is to inhibit flow.

Since this segment had no flow, the compliance calculated in the first approximation method of ALFREY was 5.1×10^{-3} cm^2/kg, because the plots of creep compliances against logarithm of the loading time made a straight line, as shown in Fig. 2. On the other hand, the retardation spectrum of the segment grown without IAA could not be decided, because there was some effect of flow even in the creep compliance obtained from recovery test, as clarified in eq. (6), that is, there was non-Newtonian flow in the segment.

In the creep test of Fig. 2, it was also shown that the creep compliance, subtracted the instantaneous creep compliance, of the segment grown without IAA was almost the same as the one of the cell wall of the segment grown with IAA. On the other hand, while the former contained the term of flow, the latter did not. Therefore, it is clear that the value of the retarded-elastic term of the compliance in the latter segment is larger than the one in the former.

From these results, it may be assumed that the application of 10 ppm IAA solution on the rapidly growing hypocotyl of black pine results in the increase of retarded elasticity and the inhibition of flow under a small load.

In connection with the sensibility of the living segment to the added load, WADA stated[2] that *Avena* coleoptile, that had stopped their extension in 0.1 mol mannitol solution, could begin their irreversible extension again under a small load. It may be, therefore, suggested that the rapid fall of stress in the preceding experiments[1] resulted from the resumption of this irreversible elongation caused by the external force. And it may also be concerned with the action of IAA for the inhibition of flow that the fall stress relaxation modulus in the living segment grown with IAA was smaller than the one in the segment grown without IAA.

References

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2) S. WAKA, Kagaku, **39**, 86 (1969). (in Japanese)